GROWTH OF THE THYROID GLAND OF *RANA PIPIENS* IN RELATION TO METAMORPHOSIS

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An exact study of the relation of the growth of the thyroid gland to metamorphosis is essential to an understanding of this process as well as of the gland itself.

Hoskins and Hoskins (1919) reported no marked growth in the thyroid of the tadpoles of *Rana sylvatica* during metamorphosis. However, Allen in the same year found considerable growth in *Bufo* thyroids at this stage. In studies on *Ambystoma*, Uhlenhuth in 1924 reported a marked growth of the thyroid and a sudden rise in the ratio of the cube root of the weight of the gland (reconstruction) to the body length at this period, followed later by a decrease in this ratio. This diversity in results may probably be due to the difficulty of securing an adequate criterion of growth.

Hoskins and Hoskins (1919) used chiefly methods of histological section and of measurement of the dissected organ but made no quantitative investigation. Allen's (1919) conclusions are based on measurements of fixed, stained and cleared specimens after dissection. In Uhlenhuth's work (1924) wax reconstructions of the glands were weighed.

Serious objection may be offered against all of these methods. In the first place, they fail to differentiate cell proliferation from colloid accumulation, and clearly the interpretation of the results depends to a great extent on this distinction. The methods of Allen and Hoskins and Hoskins are further subject to the criticism that the product of the three diameters of the gland as a measure of its volume is precarious in an organ that changes its shape and compactness as does the thyroid during metamorphosis. Variations in shrinkage that occur in the preparation of the specimen for this method further militate against its success. The method of reconstruction, on the other hand, is so laborious as to render a sufficient accumulation of evidence very difficult. These objections were distinctly realized by Hoskins and Hoskins but not overcome.

The author therefore felt that an adequate quantitative study can not make use of any of these methods. The method adopted in the present study was to count the cells in serially sectioned glands and determine the colloid volume by the use of a planimeter. This is perfectly feasible, since smaller glands involve only a few thousand cells and in larger glands not every section need be counted.

Material and Methods

Tadpoles of *Rana pipiens* were used. All specimens were derived from the same small pond in the fish hatchery near the campus at Ithaca. The identification of the tadpoles was checked by Prof. A. H. Wright.

The tissues were fixed in Bouin's fluid, sectioned serially at 10 μ and stained with hæmatoxylin and eosin.

In taking measurements, the body length of the tadpoles was taken as snout to anus minus the length of the anal canal. This made it comparable to body length in frogs.

In making the cell counts an Edinger projection machine was used, throwing an image of 650 diameters magnification. The colloid masses were first outlined, and using this outline as a guide, the cells could be easily counted. In the larger sections it was found advisable to use a further guide by placing two metal strips upon the image. These could be separated, giving a small boxed-off area for counting. By bringing the upper strip down into contact with the lower and then moving the latter, another area immediately below the preceding could be seen. In this way a large section could be accurately counted. A constant play was maintained upon the fine adjustment of the microscope in order to bring out all planes of the section. In making the counts the general rule was followed that where the sections showed over 500 cells every fifth section was counted; over 250 every third section; over 100 every other section and less than 100 every section. It was found that successive sections were so similar in cell count except at the extreme tips of the gland that this rule gave sufficient accuracy. Successive measurements of colloid mass were not nearly so uniform, which may account for the somewhat greater variations in these figures. It must be said that the colloid was so vague and indefinite in character in some sections of the first stage (stage *H*) as to make an accurate determination impossible. The results for specimen No. 54 were calculated on the basis of only one of the two thyroid lobes, since part of the other lobe had been lost. In the graph the point for this specimen is therefore recorded as a circle.

In making the calculation, the figures for the uncounted sections were interpolated and the whole added. All calculations were made by machine and checked either by repetition on the machine or with a slide rule.

To test the reliability of this method, three determinations were made of the same thyroid lobe. Though for a given section the cell counts or colloid volume varied as much as 15 per cent (average about five per cent), these variations tended to balance out in the result for an entire lobe and therefore the three determinations did not vary by more than 2 per cent for either cell count or colloid volume. This indicates an accuracy well within the normal range of variation.

In determining the dry weights given in Table II, the ordinary chemical procedure was used with the following added precautions. The Stage 2 tadpoles were treated as follows. Their tails were cut off and the bodies dissected in the dried and weighed crucible. The intestine was removed completely, drained into the crucible and laid on paper. By running a needle down for pressure, the entire contents could be removed and the intestine then returned to the crucible. The instruments were wiped on the crucible cover, and the ordinary procedure then followed. Thus only an insignificant amount of tissue fluid was lost and the intestinal contents were eliminated. Although the intestines of Stage 5 animals were found to be empty, they were similarly treated in order to equalize the losses.

Results

Table I shows a summary of results and calculations.

It was found advisable to classify the tadpoles into seven stages which may be characterized as follows:

Stage H: Half-grown tadpoles, body length about 13 mm. and total length about 33 mm. At this stage the thyroid cells occur as small groups somewhat scattered, usually enclosing a colloid mass. Their appearance varies from squamous to low cuboidal.

Stage 1: Beginning of metamorphosis. Body length about 18 mm. Hind legs as buds. Thyroid shows beginnings of definite folliculation. Epithelium squamous to low cuboidal.

Stage 2: Hind legs show growth, are 6–8 mm. in length, body length about 23 mm. Follicular nature of thyroid definitely developed. Epithelium somewhat higher than in Stage 1.

Stage 3: Hind legs about 15–23 mm. Forelegs discernible under operculum. Body about 23 mm. Marked increase in size of thyroid. Epithelium cuboidal.

Stage 4: Hind legs about 26 mm. Forelegs about 10 mm. Body length about 21 mm. Transformation of mouth (and intestine) and resorption of tail taking place. Further growth of thyroid and distension of follicles with colloid. The epithelium generally cuboidal or low columnar. During this stage the tadpole takes no food.

Stage 5: Metamorphosis recently completed, tail stub noticeable. Adult body form. Hind legs about 31 mm. Body somewhat smaller than in Stage 4. The epithelium in the thyroid much reduced in height, generally to low cuboidal or squamous.

Stage P: Post metamorphosis. Hind legs proportionately longer and body much larger than in Stage 5. These showed considerable variation in the height of the thyroid epithelium, some follicles being squamous and others cuboidal.

From Table I it can be seen that the thyroid grows enormously in both cell number and colloid volume during this period. Allen (1919) has assumed that the growth curve of the hind legs is rectilinear, in which case the growth of the gland as given in Table I would be essentially a growth curve of sigmoid form, with the period of most rapid growth in Stage 3 and periods of less activity before and after. It is not necessary, however, to make this assumption to derive the essential significance of these figures, for it can be seen that in proportion to the growth of the animal (as measured by body or hind leg length) the thyroid increases greatly and that the major portion of this increase occurs during the early growth of the hind legs. It is interesting to note that this growth becomes marked coincidently with the sudden growth of the hind legs.

The last column of Table I shows the ratio of the colloid volume to cell count. These figures are interesting in that they show a marked rise during the early stages of metamorphosis and assume a more or less constant ratio in the fourth stage. This might be taken to indicate that the thyroid hormone is not of great importance for the early stages of metamorphosis since it then tends to be stored, or alternatively that the colloid is not simply a storage place for the hormone, but rather takes some active physiological rôle in preparing the hormone for the body. A combination of both factors may operate.

Evidence for the first explanation is found by calculating the ratio of the cell count (or colloid volume) to body length cubed, as is done in the seventh and eighth columns of Table I. By dividing the cell count (or colloid volume) by the body length cubed, we get a measure of the amount of thyroid per unit volume of body.

However, caution must be observed in interpreting these figures, for it is obvious that the body length cubed is a valid measure of the amount of tissue only when the shape, proportions and nature of the tissues remain the same. This condition is fairly well satisfied by the tadpoles until the beginning of Stage 4. In this stage the tail is resorbed and the animal assumes the typical frog shape. This makes it impossible to interpret accurately the figures for this stage.

TABLE I

Preparation No.	Stage of Development	Body Length *mm.*	Hind Leg Length *mm.*	Cell Count	Volume of Colloid mm^3. × 10,000	$\frac{\text{Cell Count}}{(\text{body length})^3}$ × 1000	$\frac{\text{Vol. Col.}}{(\text{body length})^3}$ × 1000	$\frac{\text{Vol. Col.}}{\text{cell count}}$ × 1000
43	H	13		3,512	1,542	1,598	702	439
44	H	14		2,062	446	751	162	217
5	1	16		3,320	1,546	812	378	467
1	1	17		3,734	2,311	760	470	618
3	1	18		6,048	2,186	1,037	374	361
16	1	20		7,756	3,528	969	441	455
4	1	22		16,106	17,132	1,513	1,609	1,064
7	2	28	6	18,052	20,974	822	955	1,162
17	2	22½	6	15,482	27,788	1,360	2,441	1,794
48	2	22	6	25,785	21,454	2,420	2,020	832
50	2	23	8	25,756	42,723	2,117	3,511	1,698
8	3	21	14	33,396	59,028	3,606	6,374	1,767
9	3	24	15	38,723	58,434	2,801	4,227	1,509
19	3	24½	17	53,409	78,726	3,633	5,352	1,478
10	3	24	23	64,623	142,346	4,675	10,297	2,202
2	4	19		33,385	72,966	4,867	10,638	2,186
15	4	22	26	49,794	137,162	4,676	12,881	2,755
14	4	22	28	60,779	131,412	5,708	12,341	2,162
11	4	21	28	63,488	143,582	6,855	15,504	2,262
12	5	22	31	59,206	140,540	5,560	13,200	2,378
13	5	21	31	50,306	109,460	5,432	11,819	2,176
21	5	21	31	51,003	147,092	5,507	15,910	2,882
52	5	23	35	72,609	131,737	5,968	10,827	1,814
53	5-P	25	41	42,390	96,507	2,713	6,176	2,277
54	5-P	28	45	46,700	142,431	2,127	6,488	3,050
59	P	32	51	54,809	160,157	1,673	4,888	2,922
60	P	41	70	121,478	267,191	1,762	3,877	2,199

The weight of the animals would be no more satisfactory for, as is well known, the animals lose about one half their weight during this stage, a loss very largely due to the elimination of intestinal contents and of water. This can be seen in Table II, which shows that the ratio of dry weight to gross weight more than doubles. Whatever may be the significance of the desiccation phenomena revealed by these figures, a unit volume or weight of tadpole tissue cannot be compared directly to one of frog tissue. The figures for the fifth stage and the post-metamorphic animals are, however, comparable.

TABLE II

Stage 2				Stage 5			
Preparation No.	Gross Weight *grams*	Dry Weight *grams*	Dry Weight / Gross Weight *per cent*	Preparation No.	Gross Weight *grams*	Dry Weight *grams*	Dry Weight / Gross Weight *per cent*
27	1.52	.088	5.8	34	.611	.089	14.6
28	1.84	.120	6.5	25	.710	.093	13.1
29	1.92	.134	7.0	36	.743	.109	14.7
30	1.10	.069	6.3	37	.742	.106	14.3
31	1.48	.094	6.4	38	1.243	.187	15.0
32	1.12	.060	5.3	40	.755	.111	14.7
Average	1.50	.094	6.2 ± .2	Average	.801	.116	14.4 ± .3

Keeping these limitations in mind, we see that the amount of thyroid (cell number or colloid volume) per unit volume of tissue increases but slightly if at all until the hind legs start growing actively. It then shows a remarkable rise, reaching a high point three or four times the original level at the beginning of resorption of the tail (fourth stage). During this stage the behavior of this ratio is problematical, but after metamorphosis it suffers an equally remarkable loss to about one third its previous value.

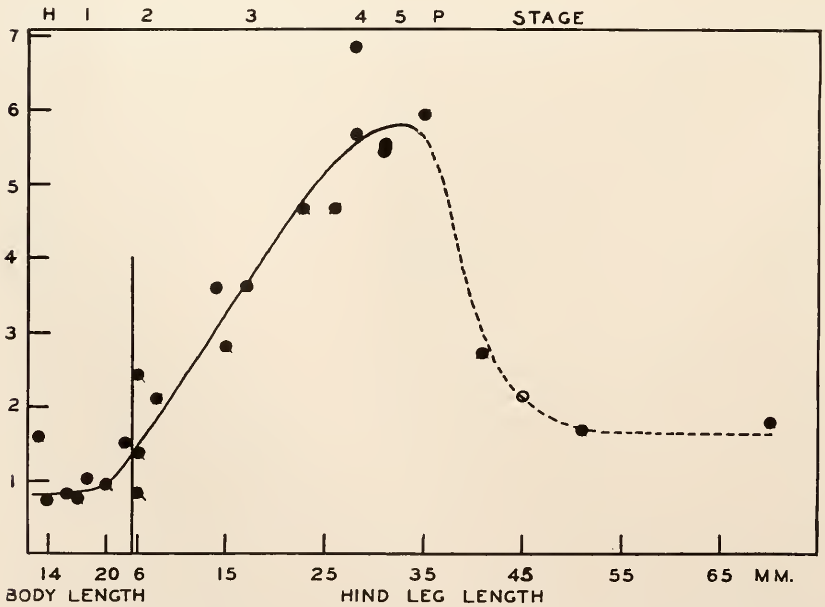

FIG. 1. Ratio of cell count to the cube of the body length plotted against body or hind leg length.

These relationships are clearly shown in the graph, Fig. 1, in which the ratio of cell count to body length cubed is plotted against the body or hind leg length. It is necessary to use body length as abscissa in the first part of the curve, for the hind legs are then but buds, whereas later the hind legs furnish the only adequate measure of the degree of development. This break in the abscissa (shown in the figure by the heavy vertical line) is not of great importance as the ratio remains practically the same until the period of the growth of the hind legs.

Since the thyroid is embryonic in structure before metamorphosis, its functional importance in the tadpole is probably small. After the beginning of metamorphosis, the amount of thyroid per unit of body rises, indicating an increasing importance. As the last stage of metamorphosis, that characterized by resorption phenomena, shows the highest ratio, it seems probable that the thyroid is of special importance in this stage. The subsequent decrease of this ratio in the frog further indicates the importance of the thyroid to the events that take place in the fourth stage.

Further evidence for this conclusion is seen in the fact mentioned above that the epithelium is highest during the fourth stage.

Furthermore, it should be noted that the growth of the colloid is essentially like that of the cell number and at no time is any loss of colloid apparent.

Summary

A quantitative study of the growth of the thyroid gland in relation to metamorphosis reveals the following facts:

1. The number of cells as counted in serial section shows an enormous increase during this process. The major part of the increase occurs between the time the hind legs start active growth and the beginning of the resorption of the tail.

2. The colloid volume behaves in a similar manner, at no time showing a sudden decrease.

3. Calculations of the ratio of cell number (or colloid volume) to the cube of the body length show that the number of cells (or colloid volume) per unit tissue rises but little before the beginning of the active growth of the hind legs. From then until the beginning of tail resorption its value more than triples. After metamorphosis has been completed, this ratio is reduced by about two thirds.

4. The ratio of the cell count to the colloid volume is found to increase four or five times from the half-grown tadpoles to the time of tail resorption. After this it tends to be constant.

These facts are interpreted as indication that the principal importance of the thyroid in metamorphosis is for the latter stages when the tail is being resorbed.

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